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(15 AND (@PY < "2003")).PGPB,USPT,USOC,EPAB,JPAB,DWPI.	11
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<u>L15</u>	L13 and cobalt	49	<u>L15</u>
<u>L14</u>	L13 and zink	0	<u>L14</u>
<u>L13</u>	L12 and aluminium	360	<u>L13</u>
<u>L12</u>	L11 and calcium	2107	<u>L12</u>
<u>L11</u>	L10 and column	2166	<u>L11</u>
<u>L10</u>	L9 and kit	2180	<u>L10</u>
<u>L9</u>	L8 and elution	2261	<u>L9</u>
<u>L8</u>	L7 and buffer	2629	<u>L8</u>
<u>L7</u>	L6 and purification	2660	<u>L7</u>
<u>L6</u>	L4 and protein	2660	<u>L6</u>

<u>L5</u>	L4 and (hard metal) same (intermediate metal)	2	<u>L5</u>
<u>L4</u>	L3 and L2	2660	<u>L4</u>
<u>L3</u>	protein purification	17345	<u>L3</u>
<u>L2</u>	metal chelate	13612	<u>L2</u>
<u>L1</u>	protein purif\$ same metal ion chelate	2	<u>L1</u>

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alerts (SDIs) affected  
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alerts (SDIs) affected  
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NEWS 13 DEC 17 THREE NEW FIELDS ADDED TO IFIPAT/IFIUDB/IFICDB  
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=> s protein purifi
=> s protein purif?
  15 FILES SEARCHED...
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  63 FILES SEARCHED...
L1      103189 PROTEIN PURIF?
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=> s metal ion chelate resin
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  56 FILES SEARCHED...
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L2      54 METAL ION CHELATE RESIN
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L3      43 L1 AND L2
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=> s L3 and hard metal ion and intermediate metal ion

21 FILES SEARCHED...

36 FILES SEARCHED...

53 FILES SEARCHED...

68 FILES SEARCHED...

L4 29 L3 AND HARD METAL ION AND INTERMEDIATE METAL ION

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L1 103189 S PROTEIN PURIF?

L2 54 S METAL ION CHELATE RESIN

L3 43 S L1 AND L2

L4 29 S L3 AND HARD METAL ION AND INTERMEDIATE METAL ION

=> d L4 1-29 bib,abs

L4 ANSWER 1 OF 29 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

AN 2003-14436 BIOTECHDS

TI New metal ion affinity peptide useful, when fused to a fusion partner polypeptide, for **protein purification** methods and to study protein-protein interactions and nucleic acid-protein interactions; vector-mediated gene transfer and expression in host cell for recombinant protein production and high throughput screening

AU TCHAGA G S; JOKHADZE G G

PA TCHAGA G S; JOKHADZE G G

PI US 2002164718 7 Nov 2002

AI US 2001-858332 15 May 2001

PRAI US 2001-858332 15 May 2001; US 1998-101867 25 Sep 1998

DT Patent

LA English

OS WPI: 2003-361747 [34]

AN 2003-14436 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - A metal ion affinity peptide (I), is new.

DETAILED DESCRIPTION - A new metal ion affinity peptide (I) consists of formula 1,2 (His-X1-X2)n1-(His-X3-X4-X5)n2-(His-X6)n3 (1) X1 and X2 = independently an amino acid with an aliphatic or an amide side chain; X1 and X2 = independently an amino acid with an aliphatic or an amide side chain; X3, X4 and X5 = independently an amino acid with a basic side chain (except His) or an acidic side chain; X6 = an amino acid with an aliphatic or an amide side chain; n1 and n2 = independently 1 - 3; and n3 = 1 - 5; (His-Asn)n (2) n = 3 - 10 (His-X1-X2)n (3) X1 and X2 = an amino acid having an acidic side chain; and n = 3 - 10. INDEPENDENT CLAIMS are also included for the following: (1) a fusion protein comprising a polypeptide fused at its amino- or carboxy-terminus to (I); (2) an isolated polynucleotide (II) comprising a nucleotide sequence encoding (I); (3) a recombinant vector (III) comprising (II); (4) a recombinant host cell comprising (III); and (5) a kit for purifying a protein, comprising (III) and a metal ion affinity resin.

BIOTECHNOLOGY - Preferred Polynucleotide: (II) comprises a nucleotide sequence that encodes a fusion protein comprising a polypeptide fused at its amino- or carboxy-terminus to (I). Preferred Kit: The kit further comprises an extraction buffer, wash buffer and an elution buffer, and a column. Preparation: No specific preparative details are given.

USE - The metal ion affinity peptide in a fusion protein allows purification of the fusion protein on a metal chelating resin. The method



involves contacting a sample comprising a fusion protein with a **metal ion chelate resin** comprising a first metal ion, preferably a **hard metal ion** such as  $\text{Fe}^{3+}$ ,  $\text{Ca}^{2+}$  and  $\text{Al}^{3+}$  and eluting any resultant bound fusion protein from the resin. The resin comprises an immobilized  $\text{Co}^{2+}$  ion. The method further comprises contacting the sample with a second immobilized metal ion affinity resin comprising a second immobilized metal ion and eluting any resultant bound fusion protein from the first and second resins. The second metal ion is an **intermediate metal ion** such as  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Co}^{2+}$  (claimed). The metal ion affinity peptide-tagged recombinant proteins are useful for the study of protein-protein interactions and nucleic acid molecule-protein interactions, using solid phase immobilized metal ion affinity chromatography (IMAC). They are also useful in high throughput systems which find use in massive parallel gene expression experiments, e.g. to determine the effect of an agent on synthesis of a protein or set of proteins, to analyze developmental stage-specific, or tissue-specific synthesis of a protein and to analyze the phosphorylation state of a protein. These methods find use in applications to characterize a protein of unknown identity or function, and in enzymatic reactions.

EXAMPLE - An affinity peptide/green fluorescent protein (GFP) fusion protein was isolated from *Escherichia coli* cells which had been transformed with the pHAT-GFPuv vector. Cell paste (0.39 g) was transferred to pre-cooled mortar, 1.2 g of alumina was added, and the mixture was ground for 2 minutes. Extraction buffer (5 ml, stored at 4degreesC) was added, after additional grinding for 2 minutes, the mixture was transferred into four eppendorph tubes. The suspension was added to the eppendorph tubes and centrifuged for 12 minutes at 12000 rotations per minute (rpm) (11750 x g). The clear supernatant was used as a starting sample for immobilized metal ion affinity chromatography (IMAC). The extraction and chromatography equilibration buffers contained 20 mM sodium phosphate buffer containing 1.0 M sodium chloride and 5 mM imidazole pH 7.0 (1 L). The elution buffer for IMAC consisted of 20 mM sodium phosphate buffer containing 1.0, M sodium chloride and 150 mM imidazole pH 7.0. Purification of the fusion protein on  $\text{Co}^{2+}$ -TALON Superflow 6 was carried out by first equilibrating the IMAC column with 5 - 10 column volumes of the equilibration buffer. The sample was loaded on the IMAC column at a flow rate of 1.0 ml per min, and 1 ml fractions were collected. The column was washed with the equilibration buffer until a baseline was reached. The adsorbed material was then eluted with elution buffer. Absorbance of each fraction at 280 nm was determined on a spectrophotometer, and protein content of each fraction also was determined. Fluorescence of each fraction was determined on a microplate reader, and the purity of the fusion protein was determined also by sodium dodecyl sulfate (SDS)-electrophoresis. Results showed that more than 95 % of the fusion protein was recovered in the fractions obtained. (23 pages)

L4 ANSWER 2 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN  
 AN 2004:759732 CAPLUS  
 DN 141:273989  
 TI Purification of fusion proteins using immobilized bi-metal affinity chromatography  
 IN Tchaga, Grigoriy S.; Jokhadze, George G.  
 PA USA  
 SO U.S. Pat. Appl. Publ., 20 pp., Cont.-in-part of U.S. Pat. Appl. 2002 164,718.  
 CODEN: USXXCO  
 DT Patent  
 LA English  
 FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2004180415	A1	20040916	US 2004-762588	20040121

	US 2002164718	A1	20021107	US 2001-858332	20010515
PRAI	US 2001-858332	A2	20010515		
	US 2003-441804P	P	20030121		
	US 1998-101867P	P	19980925		
	US 1999-404017	B2	19990923		

AB The present invention relates to IMAC (Immobilized Metal Affinity Chromatog.). The present invention provides methods of purifying proteins that include a metal ion affinity peptide. The methods generally involve contacting a fusion protein that includes a metal ion affinity peptide with at least two different metal ion chelating resins. In certain representative embodiments, the methods include contacting a fusion protein with a first **metal ion chelate resin** having a first immobilized metal ion; eluting any bound protein from the first **metal ion chelate resin**, to produce an eluate; contacting the eluate with a second **metal ion chelate resin** having a second immobilized metal ion; and eluting any bound protein from the second **metal ion chelate resin**. Also provided are kits for use in practicing the subject methods. An illustrative purification protocol for Bi-MAC (Bi-Metal Affinity Chromatog.) is shown. The subject methods find use in a variety of **protein purifn.** applications.

L4 ANSWER 3 OF 29 DGENE COPYRIGHT 2005 The Thomson Corp on STN  
 AN ABU08461 peptide DGENE  
 TI New metal ion affinity peptide useful, when fused to a fusion partner polypeptide, for **protein purification** methods and to study protein-protein interactions and nucleic acid-protein interactions

IN Tchaga G S; Jokhadze G G  
 PA (TCHA-I) TCHAGA G S.  
 (JOKH-I) JOKHADZE G G.

PI	US 2002164718	A1	20021107	23p
AI	US 2001-858332		20010515	
PRAI	US 1998-101867P		19980925	
	US 1999-404017		19990923	

DT Patent

LA English

OS 2003-361747 [34]

DESC Affinity peptide #4.

AN ABU08461 peptide DGENE

AB The present invention relates to metal ion affinity peptides, fusion proteins containing metal ion affinity peptides, and polynucleotide sequences encoding the fusion proteins. The presence of a metal ion affinity peptide in a fusion protein allows purification of the fusion protein on a metal chelating resin. The method involves contacting a sample comprising a fusion protein with a **metal ion chelate resin** comprising a first metal ion, preferably a **hard metal ion** such as Fe<sup>3+</sup>, Ca<sup>2+</sup> or Al<sup>3+</sup> and eluting any resultant bound fusion protein from the resin. The resin comprises an immobilised Co<sup>2+</sup> ion. The method further comprises contacting the sample with a second immobilised metal ion affinity resin comprising a second immobilised metal ion and eluting any resultant bound fusion protein from the first and second resins. The second metal ion is an **intermediate metal ion** such as Cu<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup> or Co<sup>2+</sup>. The metal ion affinity peptide-tagged recombinant proteins are useful for the study of protein-protein interactions and nucleic acid molecule-protein interactions, using solid phase immobilised metal ion affinity chromatography (IMAC). They are also useful in high throughput systems which find use in massive parallel gene expression experiments, e.g. to determine the effect of an agent on synthesis of a protein or set of proteins, to analyse developmental stage-specific, or tissue-specific synthesis of a protein and to analyse the phosphorylation state of a protein. These methods find use in applications to

characterise a protein of unknown identity or function, and in enzymatic reactions. The present sequence represents an affinity peptide.

L4 ANSWER 4 OF 29 DGENE COPYRIGHT 2005 The Thomson Corp on STN  
AN ABU08460 peptide DGENE  
TI New metal ion affinity peptide useful, when fused to a fusion partner polypeptide, for **protein purification** methods and to study protein-protein interactions and nucleic acid-protein interactions

IN Tchaga G S; Jokhadze G G

PA (TCHA-I) TCHAGA G S.

(JOKH-I) JOKHADZE G G.

PI US 2002164718 A1 20021107 23p

AI US 2001-858332 20010515

PRAI US 1998-101867P 19980925

US 1999-404017 19990923

DT Patent

LA English

OS 2003-361747 [34]

DESC Affinity peptide #3.

AN ABU08460 peptide DGENE

AB The present invention relates to metal ion affinity peptides, fusion proteins containing metal ion affinity peptides, and polynucleotide sequences encoding the fusion proteins. The presence of a metal ion affinity peptide in a fusion protein allows purification of the fusion protein on a metal chelating resin. The method involves contacting a sample comprising a fusion protein with a **metal ion chelate resin** comprising a first metal ion, preferably a **hard metal ion** such as Fe<sup>3+</sup>, Ca<sup>2+</sup> or Al<sup>3+</sup> and eluting any resultant bound fusion protein from the resin. The resin comprises an immobilised Co<sup>2+</sup> ion. The method further comprises contacting the sample with a second immobilised metal ion affinity resin comprising a second immobilised metal ion and eluting any resultant bound fusion protein from the first and second resins. The second metal ion is an **intermediate metal ion** such as Cu<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup> or Co<sup>2+</sup>. The metal ion affinity peptide-tagged recombinant proteins are useful for the study of protein-protein interactions and nucleic acid molecule-protein interactions, using solid phase immobilised metal ion affinity chromatography (IMAC). They are also useful in high throughput systems which find use in massive parallel gene expression experiments, e.g. to determine the effect of an agent on synthesis of a protein or set of proteins, to analyse developmental stage-specific, or tissue-specific synthesis of a protein and to analyse the phosphorylation state of a protein. These methods find use in applications to characterise a protein of unknown identity or function, and in enzymatic reactions. The present sequence represents an affinity peptide.

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AN ABU08459 peptide DGENE

TI New metal ion affinity peptide useful, when fused to a fusion partner polypeptide, for **protein purification** methods and to study protein-protein interactions and nucleic acid-protein interactions

IN Tchaga G S; Jokhadze G G

PA (TCHA-I) TCHAGA G S.

(JOKH-I) JOKHADZE G G.

PI US 2002164718 A1 20021107 23p

AI US 2001-858332 20010515

PRAI US 1998-101867P 19980925

US 1999-404017 19990923

DT Patent

LA English

OS 2003-361747 [34]

DESC Peptide cleavage site for renin.

AN ABU08459 peptide DGENE  
 AB The present invention relates to metal ion affinity peptides, fusion proteins containing metal ion affinity peptides, and polynucleotide sequences encoding the fusion proteins. The presence of a metal ion affinity peptide in a fusion protein allows purification of the fusion protein on a metal chelating resin. The method involves contacting a sample comprising a fusion protein with a **metal ion chelate resin** comprising a first metal ion, preferably a **hard metal ion** such as Fe<sup>3+</sup>, Ca<sup>2+</sup> or Al<sup>3+</sup> and eluting any resultant bound fusion protein from the resin. The resin comprises an immobilised Co<sup>2+</sup> ion. The method further comprises contacting the sample with a second immobilised metal ion affinity resin comprising a second immobilised metal ion and eluting any resultant bound fusion protein from the first and second resins. The second metal ion is an **intermediate metal ion** such as Cu<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup> or Co<sup>2+</sup>. The metal ion affinity peptide-tagged recombinant proteins are useful for the study of protein-protein interactions and nucleic acid molecule-protein interactions, using solid phase immobilised metal ion affinity chromatography (IMAC). They are also useful in high throughput systems which find use in massive parallel gene expression experiments, e.g. to determine the effect of an agent on synthesis of a protein or set of proteins, to analyse developmental stage-specific, or tissue-specific synthesis of a protein and to analyse the phosphorylation state of a protein. These methods find use in applications to characterise a protein of unknown identity or function, and in enzymatic reactions. The present sequence represents the cleavage site for renin.

L4 ANSWER 6 OF 29 DGENE COPYRIGHT 2005 The Thomson Corp on STN

AN ABU08458 peptide DGENE

TI New metal ion affinity peptide useful, when fused to a fusion partner polypeptide, for **protein purification** methods and to study protein-protein interactions and nucleic acid-protein interactions

IN Tchaga G S; Jokhadze G G

PA (TCHA-I) TCHAGA G S.

(JOKH-I) JOKHADZE G G.

PI US 2002164718 A1 20021107 23p

AI US 2001-858332 20010515

PRAI US 1998-101867P 19980925

US 1999-404017 19990923

DT Patent

LA English

OS 2003-361747 [34]

DESC Peptide cleavage site for thrombin.

AN ABU08458 peptide DGENE

AB The present invention relates to metal ion affinity peptides, fusion proteins containing metal ion affinity peptides, and polynucleotide sequences encoding the fusion proteins. The presence of a metal ion affinity peptide in a fusion protein allows purification of the fusion protein on a metal chelating resin. The method involves contacting a sample comprising a fusion protein with a **metal ion chelate resin** comprising a first metal ion, preferably a **hard metal ion** such as Fe<sup>3+</sup>, Ca<sup>2+</sup> or Al<sup>3+</sup> and eluting any resultant bound fusion protein from the resin. The resin comprises an immobilised Co<sup>2+</sup> ion. The method further comprises contacting the sample with a second immobilised metal ion affinity resin comprising a second immobilised metal ion and eluting any resultant bound fusion protein from the first and second resins. The second metal ion is an **intermediate metal ion** such as Cu<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup> or Co<sup>2+</sup>. The metal ion affinity peptide-tagged recombinant proteins are useful for the study of protein-protein interactions and nucleic acid molecule-protein interactions, using solid phase immobilised metal ion affinity chromatography (IMAC). They are also useful in high throughput systems which find use in massive parallel gene expression

experiments, e.g. to determine the effect of an agent on synthesis of a protein or set of proteins, to analyse developmental stage-specific, or tissue-specific synthesis of a protein and to analyse the phosphorylation state of a protein. These methods find use in applications to characterise a protein of unknown identity or function, and in enzymatic reactions. The present sequence represents the cleavage site for thrombin.

L4 ANSWER 7 OF 29 DGENE COPYRIGHT 2005 The Thomson Corp on STN  
 AN ABU08457 peptide DGENE  
 TI New metal ion affinity peptide useful, when fused to a fusion partner polypeptide, for **protein purification** methods and to study protein-protein interactions and nucleic acid-protein interactions  
 -  
 IN Tchaga G S; Jokhadze G G  
 PA (TCHA-I) TCHAGA G S.  
 (JOKH-I) JOKHADZE G G.  
 PI US 2002164718 A1 20021107 23p  
 AI US 2001-858332 20010515  
 PRAI US 1998-101867P 19980925  
 US 1999-404017 19990923  
 DT Patent  
 LA English  
 OS 2003-361747 [34]  
 DESC Peptide cleavage site for factor Xa.  
 AN ABU08457 peptide DGENE  
 AB The present invention relates to metal ion affinity peptides, fusion proteins containing metal ion affinity peptides, and polynucleotide sequences encoding the fusion proteins. The presence of a metal ion affinity peptide in a fusion protein allows purification of the fusion protein on a metal chelating resin. The method involves contacting a sample comprising a fusion protein with a **metal ion chelate resin** comprising a first metal ion, preferably a **hard metal ion** such as Fe<sup>3+</sup>, Ca<sup>2+</sup> or Al<sup>3+</sup> and eluting any resultant bound fusion protein from the resin. The resin comprises an immobilised Co<sup>2+</sup> ion. The method further comprises contacting the sample with a second immobilised metal ion affinity resin comprising a second immobilised metal ion and eluting any resultant bound fusion protein from the first and second resins. The second metal ion is an **intermediate metal ion** such as Cu<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup> or Co<sup>2+</sup>. The metal ion affinity peptide-tagged recombinant proteins are useful for the study of protein-protein interactions and nucleic acid molecule-protein interactions, using solid phase immobilised metal ion affinity chromatography (IMAC). They are also useful in high throughput systems which find use in massive parallel gene expression experiments, e.g. to determine the effect of an agent on synthesis of a protein or set of proteins, to analyse developmental stage-specific, or tissue-specific synthesis of a protein and to analyse the phosphorylation state of a protein. These methods find use in applications to characterise a protein of unknown identity or function, and in enzymatic reactions. The present sequence represents the cleavage site for factor Xa.

L4 ANSWER 8 OF 29 DGENE COPYRIGHT 2005 The Thomson Corp on STN  
 AN ABU08456 peptide DGENE  
 TI New metal ion affinity peptide useful, when fused to a fusion partner polypeptide, for **protein purification** methods and to study protein-protein interactions and nucleic acid-protein interactions  
 -  
 IN Tchaga G S; Jokhadze G G  
 PA (TCHA-I) TCHAGA G S.  
 (JOKH-I) JOKHADZE G G.  
 PI US 2002164718 A1 20021107 23p  
 AI US 2001-858332 20010515

PRAI US 1998-101867P 19980925  
US 1999-404017 19990923

DT Patent

LA English

OS 2003-361747 [34]

DESC c-myc tag peptide.

AN ABU08456 peptide DGENE

AB The present invention relates to metal ion affinity peptides, fusion proteins containing metal ion affinity peptides, and polynucleotide sequences encoding the fusion proteins. The presence of a metal ion affinity peptide in a fusion protein allows purification of the fusion protein on a metal chelating resin. The method involves contacting a sample comprising a fusion protein with a **metal ion chelate resin** comprising a first metal ion, preferably a **hard metal ion** such as Fe<sup>3+</sup>, Ca<sup>2+</sup> or Al<sup>3+</sup> and eluting any resultant bound fusion protein from the resin. The resin comprises an immobilised Co<sup>2+</sup> ion. The method further comprises contacting the sample with a second immobilised metal ion affinity resin comprising a second immobilised metal ion and eluting any resultant bound fusion protein from the first and second resins. The second metal ion is an **intermediate metal ion** such as Cu<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup> or Co<sup>2+</sup>. The metal ion affinity peptide-tagged recombinant proteins are useful for the study of protein-protein interactions and nucleic acid molecule-protein interactions, using solid phase immobilised metal ion affinity chromatography (IMAC). They are also useful in high throughput systems which find use in massive parallel gene expression experiments, e.g. to determine the effect of an agent on synthesis of a protein or set of proteins, to analyse developmental stage-specific, or tissue-specific synthesis of a protein and to analyse the phosphorylation state of a protein. These methods find use in applications to characterise a protein of unknown identity or function, and in enzymatic reactions. The present sequence represents c-myc tag peptide.

L4 ANSWER 9 OF 29 DGENE COPYRIGHT 2005 The Thomson Corp on STN

AN ABU08455 peptide DGENE

TI New metal ion affinity peptide useful, when fused to a fusion partner polypeptide, for **protein purification** methods and to study protein-protein interactions and nucleic acid-protein interactions

IN Tchaga G S; Jokhadze G G

PA (TCHA-I) TCHAGA G S.  
(JOKH-I) JOKHADZE G G.

PI US 2002164718 A1 20021107 23p

AI US 2001-858332 20010515

PRAI US 1998-101867P 19980925

US 1999-404017 19990923

DT Patent

LA English

OS 2003-361747 [34]

DESC FLAG tag peptide.

AN ABU08455 peptide DGENE

AB The present invention relates to metal ion affinity peptides, fusion proteins containing metal ion affinity peptides, and polynucleotide sequences encoding the fusion proteins. The presence of a metal ion affinity peptide in a fusion protein allows purification of the fusion protein on a metal chelating resin. The method involves contacting a sample comprising a fusion protein with a **metal ion chelate resin** comprising a first metal ion, preferably a **hard metal ion** such as Fe<sup>3+</sup>, Ca<sup>2+</sup> or Al<sup>3+</sup> and eluting any resultant bound fusion protein from the resin. The resin comprises an immobilised Co<sup>2+</sup> ion. The method further comprises contacting the sample with a second immobilised metal ion affinity resin comprising a second immobilised metal ion and eluting any resultant bound fusion protein from the first and second resins. The second metal ion is

an **intermediate metal ion** such as Cu<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup> or Co<sup>2+</sup>. The metal ion affinity peptide-tagged recombinant proteins are useful for the study of protein-protein interactions and nucleic acid molecule-protein interactions, using solid phase immobilised metal ion affinity chromatography (IMAC). They are also useful in high throughput systems which find use in massive parallel gene expression experiments, e.g. to determine the effect of an agent on synthesis of a protein or set of proteins, to analyse developmental stage-specific, or tissue-specific synthesis of a protein and to analyse the phosphorylation state of a protein. These methods find use in applications to characterise a protein of unknown identity or function, and in enzymatic reactions. The present sequence represents FLAG tag peptide.

L4 ANSWER 10 OF 29 DGENE COPYRIGHT 2005 The Thomson Corp on STN  
 AN ABU08454 peptide DGENE  
 TI New metal ion affinity peptide useful, when fused to a fusion partner polypeptide, for **protein purification** methods and to study protein-protein interactions and nucleic acid-protein interactions

IN Tchaga G S; Jokhadze G G

PA (TCHA-I) TCHAGA G S.  
 (JOKH-I) JOKHADZE G G.

PI US 2002164718 A1 20021107 23p

AI US 2001-858332 20010515

PRAI US 1998-101867P 19980925

US 1999-404017 19990923

DT Patent

LA English

OS 2003-361747 [34]

DESC Haemagglutinin (HA) tag peptide.

AN ABU08454 peptide DGENE

AB The present invention relates to metal ion affinity peptides, fusion proteins containing metal ion affinity peptides, and polynucleotide sequences encoding the fusion proteins. The presence of a metal ion affinity peptide in a fusion protein allows purification of the fusion protein on a metal chelating resin. The method involves contacting a sample comprising a fusion protein with a **metal ion chelate resin** comprising a first metal ion, preferably a **hard metal ion** such as Fe<sup>3+</sup>, Ca<sup>2+</sup> or Al<sup>3+</sup> and eluting any resultant bound fusion protein from the resin. The resin comprises an immobilised Co<sup>2+</sup> ion. The method further comprises contacting the sample with a second immobilised metal ion affinity resin comprising a second immobilised metal ion and eluting any resultant bound fusion protein from the first and second resins. The second metal ion is an **intermediate metal ion** such as Cu<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup> or Co<sup>2+</sup>. The metal ion affinity peptide-tagged recombinant proteins are useful for the study of protein-protein interactions and nucleic acid molecule-protein interactions, using solid phase immobilised metal ion affinity chromatography (IMAC). They are also useful in high throughput systems which find use in massive parallel gene expression experiments, e.g. to determine the effect of an agent on synthesis of a protein or set of proteins, to analyse developmental stage-specific, or tissue-specific synthesis of a protein and to analyse the phosphorylation state of a protein. These methods find use in applications to characterise a protein of unknown identity or function, and in enzymatic reactions. The present sequence represents haemagglutinin (HA) tag peptide.

L4 ANSWER 11 OF 29 DGENE COPYRIGHT 2005 The Thomson Corp on STN

AN ABU08453 peptide DGENE

TI New metal ion affinity peptide useful, when fused to a fusion partner polypeptide, for **protein purification** methods and to study protein-protein interactions and nucleic acid-protein interactions

IN Tchaga G S; Jokhadze G G  
 PA (TCHA-I) TCHAGA G S.  
 (JOKH-I) JOKHADZE G G.  
 PI US 2002164718 A1 20021107 23p  
 AI US 2001-858332 20010515  
 PRAI US 1998-101867P 19980925  
 US 1999-404017 19990923  
 DT Patent  
 LA English  
 OS 2003-361747 [34]  
 DESC Affinity peptide #2.  
 AN ABU08453 peptide DGENE  
 AB The present invention relates to metal ion affinity peptides, fusion proteins containing metal ion affinity peptides, and polynucleotide sequences encoding the fusion proteins. The presence of a metal ion affinity peptide in a fusion protein allows purification of the fusion protein on a metal chelating resin. The method involves contacting a sample comprising a fusion protein with a **metal ion chelate resin** comprising a first metal ion, preferably a **hard metal ion** such as Fe<sup>3+</sup>, Ca<sup>2+</sup> or Al<sup>3+</sup> and eluting any resultant bound fusion protein from the resin. The resin comprises an immobilised Co<sup>2+</sup> ion. The method further comprises contacting the sample with a second immobilised metal ion affinity resin comprising a second immobilised metal ion and eluting any resultant bound fusion protein from the first and second resins. The second metal ion is an **intermediate metal ion** such as Cu<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup> or Co<sup>2+</sup>. The metal ion affinity peptide-tagged recombinant proteins are useful for the study of protein-protein interactions and nucleic acid molecule-protein interactions, using solid phase immobilised metal ion affinity chromatography (IMAC). They are also useful in high throughput systems which find use in massive parallel gene expression experiments, e.g. to determine the effect of an agent on synthesis of a protein or set of proteins, to analyse developmental stage-specific, or tissue-specific synthesis of a protein and to analyse the phosphorylation state of a protein. These methods find use in applications to characterise a protein of unknown identity or function, and in enzymatic reactions. The present sequence represents an affinity peptide.

L4 ANSWER 12 OF 29 DGENE COPYRIGHT 2005 The Thomson Corp on STN  
 AN ABU08452 peptide DGENE  
 TI New metal ion affinity peptide useful, when fused to a fusion partner polypeptide, for **protein purification** methods and to study protein-protein interactions and nucleic acid-protein interactions

IN Tchaga G S; Jokhadze G G  
 PA (TCHA-I) TCHAGA G S.  
 (JOKH-I) JOKHADZE G G.  
 PI US 2002164718 A1 20021107 23p  
 AI US 2001-858332 20010515  
 PRAI US 1998-101867P 19980925  
 US 1999-404017 19990923  
 DT Patent  
 LA English  
 OS 2003-361747 [34]  
 DESC Affinity peptide #1.  
 AN ABU08452 peptide DGENE  
 AB The present invention relates to metal ion affinity peptides, fusion proteins containing metal ion affinity peptides, and polynucleotide sequences encoding the fusion proteins. The presence of a metal ion affinity peptide in a fusion protein allows purification of the fusion protein on a metal chelating resin. The method involves contacting a sample comprising a fusion protein with a **metal ion chelate resin** comprising a first metal ion, preferably a **hard metal ion** such as Fe<sup>3+</sup>, Ca<sup>2+</sup> or Al<sup>3+</sup>



and eluting any resultant bound fusion protein from the resin. The resin comprises an immobilised  $\text{Co}^{2+}$  ion. The method further comprises contacting the sample with a second immobilised metal ion affinity resin comprising a second immobilised metal ion and eluting any resultant bound fusion protein from the first and second resins. The second metal ion is an **intermediate metal ion** such as  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$  or  $\text{Co}^{2+}$ . The metal ion affinity peptide-tagged recombinant proteins are useful for the study of protein-protein interactions and nucleic acid molecule-protein interactions, using solid phase immobilised metal ion affinity chromatography (IMAC). They are also useful in high throughput systems which find use in massive parallel gene expression experiments, e.g. to determine the effect of an agent on synthesis of a protein or set of proteins, to analyse developmental stage-specific, or tissue-specific synthesis of a protein and to analyse the phosphorylation state of a protein. These methods find use in applications to characterise a protein of unknown identity or function, and in enzymatic reactions. The present sequence represents an affinity peptide.

L4 ANSWER 13 OF 29 DGENE COPYRIGHT 2005 The Thomson Corp on STN  
 AN ABU08451 peptide DGENE  
 TI New metal ion affinity peptide useful, when fused to a fusion partner polypeptide, for **protein purification** methods and to study protein-protein interactions and nucleic acid-protein interactions

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IN Tchaga G S; Jokhadze G G  
 PA (TCHA-I) TCHAGA G S.  
 (JOKH-I) JOKHADZE G G.  
 PI US 2002164718 A1 20021107 23p  
 AI US 2001-858332 20010515  
 PRAI US 1998-101867P 19980925  
 US 1999-404017 19990923  
 DT Patent  
 LA English  
 OS 2003-361747 [34]  
 DESC Peptide cleavage site for enterokinase.  
 AN ABU08451 peptide DGENE  
 AB The present invention relates to metal ion affinity peptides, fusion proteins containing metal ion affinity peptides, and polynucleotide sequences encoding the fusion proteins. The presence of a metal ion affinity peptide in a fusion protein allows purification of the fusion protein on a metal chelating resin. The method involves contacting a sample comprising a fusion protein with a **metal ion chelate resin** comprising a first metal ion, preferably a **hard metal ion** such as  $\text{Fe}^{3+}$ ,  $\text{Ca}^{2+}$  or  $\text{Al}^{3+}$  and eluting any resultant bound fusion protein from the resin. The resin comprises an immobilised  $\text{Co}^{2+}$  ion. The method further comprises contacting the sample with a second immobilised metal ion affinity resin comprising a second immobilised metal ion and eluting any resultant bound fusion protein from the first and second resins. The second metal ion is an **intermediate metal ion** such as  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$  or  $\text{Co}^{2+}$ . The metal ion affinity peptide-tagged recombinant proteins are useful for the study of protein-protein interactions and nucleic acid molecule-protein interactions, using solid phase immobilised metal ion affinity chromatography (IMAC). They are also useful in high throughput systems which find use in massive parallel gene expression experiments, e.g. to determine the effect of an agent on synthesis of a protein or set of proteins, to analyse developmental stage-specific, or tissue-specific synthesis of a protein and to analyse the phosphorylation state of a protein. These methods find use in applications to characterise a protein of unknown identity or function, and in enzymatic reactions. The present sequence represents the cleavage site for enterokinase.

L4 ANSWER 14 OF 29 DGENE COPYRIGHT 2005 The Thomson Corp on STN

AN ABU08450 peptide DGENE  
TI New metal ion affinity peptide useful, when fused to a fusion partner polypeptide, for **protein purification** methods and to study protein-protein interactions and nucleic acid-protein interactions

IN Tchaga G S; Jokhadze G G

PA (TCHA-I) TCHAGA G S.  
(JOKH-I) JOKHADZE G G.

PI US 2002164718 A1 20021107 23p

AI US 2001-858332 20010515

PRAI US 1998-101867P 19980925

US 1999-404017 19990923

DT Patent

LA English

OS 2003-361747 [34]

CR N-PSDB: ABX94283

DESC Affinity purification peptide #5.

AN ABU08450 peptide DGENE

AB The present invention relates to metal ion affinity peptides, fusion proteins containing metal ion affinity peptides, and polynucleotide sequences encoding the fusion proteins. The presence of a metal ion affinity peptide in a fusion protein allows purification of the fusion protein on a metal chelating resin. The method involves contacting a sample comprising a fusion protein with a **metal ion chelate resin** comprising a first metal ion, preferably a **hard metal ion** such as Fe<sup>3+</sup>, Ca<sup>2+</sup> or Al<sup>3+</sup> and eluting any resultant bound fusion protein from the resin. The resin comprises an immobilised Co<sup>2+</sup> ion. The method further comprises contacting the sample with a second immobilised metal ion affinity resin comprising a second immobilised metal ion and eluting any resultant bound fusion protein from the first and second resins. The second metal ion is an **intermediate metal ion** such as Cu<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup> or Co<sup>2+</sup>. The metal ion affinity peptide-tagged recombinant proteins are useful for the study of protein-protein interactions and nucleic acid molecule-protein interactions, using solid phase immobilised metal ion affinity chromatography (IMAC). They are also useful in high throughput systems which find use in massive parallel gene expression experiments, e.g. to determine the effect of an agent on synthesis of a protein or set of proteins, to analyse developmental stage-specific, or tissue-specific synthesis of a protein and to analyse the phosphorylation state of a protein. These methods find use in applications to characterise a protein of unknown identity or function, and in enzymatic reactions. ABU08446-ABU08450 represent affinity purification peptides.

L4 ANSWER 15 OF 29 DGENE COPYRIGHT 2005 The Thomson Corp on STN

AN ABU08449 peptide DGENE

TI New metal ion affinity peptide useful, when fused to a fusion partner polypeptide, for **protein purification** methods and to study protein-protein interactions and nucleic acid-protein interactions

IN Tchaga G S; Jokhadze G G

PA (TCHA-I) TCHAGA G S.  
(JOKH-I) JOKHADZE G G.

PI US 2002164718 A1 20021107 23p

AI US 2001-858332 20010515

PRAI US 1998-101867P 19980925

US 1999-404017 19990923

DT Patent

LA English

OS 2003-361747 [34]

CR N-PSDB: ABX94282

DESC Affinity purification peptide #4.

AN ABU08449 peptide DGENE

AB The present invention relates to metal ion affinity peptides, fusion

proteins containing metal ion affinity peptides, and polynucleotide sequences encoding the fusion proteins. The presence of a metal ion affinity peptide in a fusion protein allows purification of the fusion protein on a metal chelating resin. The method involves contacting a sample comprising a fusion protein with a **metal ion chelate resin** comprising a first metal ion, preferably a **hard metal ion** such as Fe<sup>3+</sup>, Ca<sup>2+</sup> or Al<sup>3+</sup> and eluting any resultant bound fusion protein from the resin. The resin comprises an immobilised Co<sup>2+</sup> ion. The method further comprises contacting the sample with a second immobilised metal ion affinity resin comprising a second immobilised metal ion and eluting any resultant bound fusion protein from the first and second resins. The second metal ion is an **intermediate metal ion** such as Cu<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup> or Co<sup>2+</sup>. The metal ion affinity peptide-tagged recombinant proteins are useful for the study of protein-protein interactions and nucleic acid molecule-protein interactions, using solid phase immobilised metal ion affinity chromatography (IMAC). They are also useful in high throughput systems which find use in massive parallel gene expression experiments, e.g. to determine the effect of an agent on synthesis of a protein or set of proteins, to analyse developmental stage-specific, or tissue-specific synthesis of a protein and to analyse the phosphorylation state of a protein. These methods find use in applications to characterise a protein of unknown identity or function, and in enzymatic reactions. ABU08446-ABU08450 represent affinity purification peptides.

L4 ANSWER 16 OF 29 DGENE COPYRIGHT 2005 The Thomson Corp on STN  
 AN ABU08448 peptide DGENE  
 TI New metal ion affinity peptide useful, when fused to a fusion partner polypeptide, for **protein purification** methods and to study protein-protein interactions and nucleic acid-protein interactions

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IN Tchaga G S; Jokhadze G G  
 PA (TCHA-I) TCHAGA G S.  
 (JOKH-I) JOKHADZE G G.  
 PI US 2002164718 A1 20021107 23p  
 AI US 2001-858332 20010515  
 PRAI US 1998-101867P 19980925  
 US 1999-404017 19990923  
 DT Patent  
 LA English  
 OS 2003-361747 [34]  
 CR N-PSDB: ABX94281  
 DESC Affinity purification peptide #3.  
 AN ABU08448 peptide DGENE  
 AB The present invention relates to metal ion affinity peptides, fusion proteins containing metal ion affinity peptides, and polynucleotide sequences encoding the fusion proteins. The presence of a metal ion affinity peptide in a fusion protein allows purification of the fusion protein on a metal chelating resin. The method involves contacting a sample comprising a fusion protein with a **metal ion chelate resin** comprising a first metal ion, preferably a **hard metal ion** such as Fe<sup>3+</sup>, Ca<sup>2+</sup> or Al<sup>3+</sup> and eluting any resultant bound fusion protein from the resin. The resin comprises an immobilised Co<sup>2+</sup> ion. The method further comprises contacting the sample with a second immobilised metal ion affinity resin comprising a second immobilised metal ion and eluting any resultant bound fusion protein from the first and second resins. The second metal ion is an **intermediate metal ion** such as Cu<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup> or Co<sup>2+</sup>. The metal ion affinity peptide-tagged recombinant proteins are useful for the study of protein-protein interactions and nucleic acid molecule-protein interactions, using solid phase immobilised metal ion affinity chromatography (IMAC). They are also useful in high throughput systems which find use in massive parallel gene expression experiments, e.g. to determine the effect of an agent on synthesis of a

protein or set of proteins, to analyse developmental stage-specific, or tissue-specific synthesis of a protein and to analyse the phosphorylation state of a protein. These methods find use in applications to characterise a protein of unknown identity or function, and in enzymatic reactions. ABU08446-ABU08450 represent affinity purification peptides.

L4 ANSWER 17 OF 29 DGENE COPYRIGHT 2005 The Thomson Corp on STN  
AN ABU08447 peptide DGENE  
TI New metal ion affinity peptide useful, when fused to a fusion partner polypeptide, for **protein purification** methods and to study protein-protein interactions and nucleic acid-protein interactions

IN Tchaga G S; Jokhadze G G

PA ~~(TCHA-I) TCHAGA G S.~~  
~~(JOKH-I) JOKHADZE G G.~~

PI US 2002164718 A1 20021107 23p

AI US 2001-858332 20010515

PRAI US 1998-101867P 19980925

US 1999-404017 19990923

DT Patent

LA English

OS 2003-361747 [34]

CR N-PSDB: ABX94280

DESC Affinity purification peptide #2.

AN ABU08447 peptide DGENE

AB The present invention relates to metal ion affinity peptides, fusion proteins containing metal ion affinity peptides, and polynucleotide sequences encoding the fusion proteins. The presence of a metal ion affinity peptide in a fusion protein allows purification of the fusion protein on a metal chelating resin. The method involves contacting a sample comprising a fusion protein with a **metal ion chelate resin** comprising a first metal ion, preferably a **hard metal ion** such as Fe<sup>3+</sup>, Ca<sup>2+</sup> or Al<sup>3+</sup> and eluting any resultant bound fusion protein from the resin. The resin comprises an immobilised Co<sup>2+</sup> ion. The method further comprises contacting the sample with a second immobilised metal ion affinity resin comprising a second immobilised metal ion and eluting any resultant bound fusion protein from the first and second resins. The second metal ion is an **intermediate metal ion** such as Cu<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup> or Co<sup>2+</sup>. The metal ion affinity peptide-tagged recombinant proteins are useful for the study of protein-protein interactions and nucleic acid molecule-protein interactions, using solid phase immobilised metal ion affinity chromatography (IMAC). They are also useful in high throughput systems which find use in massive parallel gene expression experiments, e.g. to determine the effect of an agent on synthesis of a protein or set of proteins, to analyse developmental stage-specific, or tissue-specific synthesis of a protein and to analyse the phosphorylation state of a protein. These methods find use in applications to characterise a protein of unknown identity or function, and in enzymatic reactions. ABU08446-ABU08450 represent affinity purification peptides.

L4 ANSWER 18 OF 29 DGENE COPYRIGHT 2005 The Thomson Corp on STN  
AN ABU08446 peptide DGENE  
TI New metal ion affinity peptide useful, when fused to a fusion partner polypeptide, for **protein purification** methods and to study protein-protein interactions and nucleic acid-protein interactions

IN Tchaga G S; Jokhadze G G

PA ~~(TCHA-I) TCHAGA G S.~~  
~~(JOKH-I) JOKHADZE G G.~~

PI US 2002164718 A1 20021107 23p

AI US 2001-858332 20010515

PRAI US 1998-101867P 19980925

US 1999-404017 19990923

DT Patent  
 LA English  
 OS 2003-361747 [34]  
 CR N-PSDB: ABX94279  
 DESC Affinity purification peptide #1.  
 AN ABU08446 peptide DGENE  
 AB The present invention relates to metal ion affinity peptides, fusion proteins containing metal ion affinity peptides, and polynucleotide sequences encoding the fusion proteins. The presence of a metal ion affinity peptide in a fusion protein allows purification of the fusion protein on a metal chelating resin. The method involves contacting a sample comprising a fusion protein with a **metal ion chelate resin** comprising a first metal ion, preferably a **hard metal ion** such as Fe<sup>3+</sup>, Ca<sup>2+</sup> or Al<sup>3+</sup> and eluting any resultant bound fusion protein from the resin. The resin comprises an immobilised Co<sup>2+</sup> ion. The method further comprises contacting the sample with a second immobilised metal ion affinity resin comprising a second immobilised metal ion and eluting any resultant bound fusion protein from the first and second resins. The second metal ion is an **intermediate metal ion** such as Cu<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup> or Co<sup>2+</sup>. The metal ion affinity peptide-tagged recombinant proteins are useful for the study of protein-protein interactions and nucleic acid molecule-protein interactions, using solid phase immobilised metal ion affinity chromatography (IMAC). They are also useful in high throughput systems which find use in massive parallel gene expression experiments, e.g. to determine the effect of an agent on synthesis of a protein or set of proteins, to analyse developmental stage-specific, or tissue-specific synthesis of a protein and to analyse the phosphorylation state of a protein. These methods find use in applications to characterise a protein of unknown identity or function, and in enzymatic reactions. ABU08446-ABU08450 represent affinity purification peptides.

L4 ANSWER 19 OF 29 DGENE COPYRIGHT 2005 The Thomson Corp on STN  
 AN ABU08445 Protein DGENE  
 TI New metal ion affinity peptide useful, when fused to a fusion partner polypeptide, for **protein purification** methods and to study protein-protein interactions and nucleic acid-protein interactions

IN Tchaga G S; Jokhadze G G

PA (TCHA-I) TCHAGA G S.  
 (JOKH-I) JOKHADZE G G.

PI US 2002164718 A1 20021107 23p

AI US 2001-858332 20010515

PRAI US 1998-101867P 19980925

US 1999-404017 19990923

DT Patent

LA English

OS 2003-361747 [34]

CR N-PSDB: ABX94278

DESC Recombinant enterokinase (EK) fusion protein encoded by vector pHAT-EK.

AN ABU08445 Protein DGENE

AB The present invention relates to metal ion affinity peptides, fusion proteins containing metal ion affinity peptides, and polynucleotide sequences encoding the fusion proteins. The presence of a metal ion affinity peptide in a fusion protein allows purification of the fusion protein on a metal chelating resin. The method involves contacting a sample comprising a fusion protein with a **metal ion chelate resin** comprising a first metal ion, preferably a **hard metal ion** such as Fe<sup>3+</sup>, Ca<sup>2+</sup> or Al<sup>3+</sup> and eluting any resultant bound fusion protein from the resin. The resin comprises an immobilised Co<sup>2+</sup> ion. The method further comprises contacting the sample with a second immobilised metal ion affinity resin comprising a second immobilised metal ion and eluting any resultant bound fusion protein from the first and second resins. The second metal ion is

an **intermediate metal ion** such as Cu<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup> or Co<sup>2+</sup>. The metal ion affinity peptide-tagged recombinant proteins are useful for the study of protein-protein interactions and nucleic acid molecule-protein interactions, using solid phase immobilised metal ion affinity chromatography (IMAC). They are also useful in high throughput systems which find use in massive parallel gene expression experiments, e.g. to determine the effect of an agent on synthesis of a protein or set of proteins, to analyse developmental stage-specific, or tissue-specific synthesis of a protein and to analyse the phosphorylation state of a protein. These methods find use in applications to characterise a protein of unknown identity or function, and in enzymatic reactions. The present sequence represents the recombinant enterokinase (EK) fusion protein encoded by vector pHAT-EK.

L4 ANSWER 20 OF 29 DGENE COPYRIGHT 2005 The Thomson Corp on STN  
 AN ABX94283 DNA DGENE  
 TI New metal ion affinity peptide useful, when fused to a fusion partner polypeptide, for **protein purification** methods and to study protein-protein interactions and nucleic acid-protein interactions

IN ~~Tchaga G S; Jokhadze G G~~

PA (TCHA-I) TCHAGA G S.

(JOKH-I) JOKHADZE G G.

PI US 2002164718 A1 20021107 23p

AI US 2001-858332 20010515

PRAI US 1998-101867P 19980925

US 1999-404017 19990923

DT Patent

LA English

OS 2003-361747 [34]

CR P-PSDB: ABU08450

DESC DNA variant sequence encoding affinity purification peptide #5.

AN ABX94283 DNA DGENE

AB The present invention relates to metal ion affinity peptides, fusion proteins containing metal ion affinity peptides, and polynucleotide sequences encoding the fusion proteins. The presence of a metal ion affinity peptide in a fusion protein allows purification of the fusion protein on a metal chelating resin. The method involves contacting a sample comprising a fusion protein with a **metal ion chelate resin** comprising a first metal ion, preferably a **hard metal ion** such as Fe<sup>3+</sup>, Ca<sup>2+</sup> or Al<sup>3+</sup> and eluting any resultant bound fusion protein from the resin. The resin comprises an immobilised Co<sup>2+</sup> ion. The method further comprises contacting the sample with a second immobilised metal ion affinity resin comprising a second immobilised metal ion and eluting any resultant bound fusion protein from the first and second resins. The second metal ion is an **intermediate metal ion** such as Cu<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup> or Co<sup>2+</sup>. The metal ion affinity peptide-tagged recombinant proteins are useful for the study of protein-protein interactions and nucleic acid molecule-protein interactions, using solid phase immobilised metal ion affinity chromatography (IMAC). They are also useful in high throughput systems which find use in massive parallel gene expression experiments, e.g. to determine the effect of an agent on synthesis of a protein or set of proteins, to analyse developmental stage-specific, or tissue-specific synthesis of a protein and to analyse the phosphorylation state of a protein. These methods find use in applications to characterise a protein of unknown identity or function, and in enzymatic reactions. ABX94279-ABX94283 represent DNA variants that encode affinity purification peptides.

L4 ANSWER 21 OF 29 DGENE COPYRIGHT 2005 The Thomson Corp on STN

AN ABX94282 DNA DGENE

TI New metal ion affinity peptide useful, when fused to a fusion partner polypeptide, for **protein purification** methods and to

# study protein-protein interactions and nucleic acid-protein interactions

IN Tchaga G S; Jokhadze G G  
PA (TCHA-I) TCHAGA G S.  
(JOKH-I) JOKHADZE G G.  
PI US 2002164718 A1 20021107 23p  
AI US 2001-858332 20010515  
PRAI US 1998-101867P 19980925  
US 1999-404017 19990923  
DT Patent  
LA English  
OS 2003-361747 [34]  
CR P-PSDB: ABU08449  
DESC DNA variant sequence encoding affinity purification peptide #4.  
AN ABX94282 DNA DGENE  
AB The present invention relates to metal ion affinity peptides, fusion proteins containing metal ion affinity peptides, and polynucleotide sequences encoding the fusion proteins. The presence of a metal ion affinity peptide in a fusion protein allows purification of the fusion protein on a metal chelating resin. The method involves contacting a sample comprising a fusion protein with a **metal ion chelate resin** comprising a first metal ion, preferably a **hard metal ion** such as Fe<sup>3+</sup>, Ca<sup>2+</sup> or Al<sup>3+</sup> and eluting any resultant bound fusion protein from the resin. The resin comprises an immobilised Co<sup>2+</sup> ion. The method further comprises contacting the sample with a second immobilised metal ion affinity resin comprising a second immobilised metal ion and eluting any resultant bound fusion protein from the first and second resins. The second metal ion is an **intermediate metal ion** such as Cu<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup> or Co<sup>2+</sup>. The metal ion affinity peptide-tagged recombinant proteins are useful for the study of protein-protein interactions and nucleic acid molecule-protein interactions, using solid phase immobilised metal ion affinity chromatography (IMAC). They are also useful in high throughput systems which find use in massive parallel gene expression experiments, e.g. to determine the effect of an agent on synthesis of a protein or set of proteins, to analyse developmental stage-specific, or tissue-specific synthesis of a protein and to analyse the phosphorylation state of a protein. These methods find use in applications to characterise a protein of unknown identity or function, and in enzymatic reactions. ABX94279-ABX94283 represent DNA variants that encode affinity purification peptides.

L4 ANSWER 22 OF 29 DGENE COPYRIGHT 2005 The Thomson Corp on STN  
AN ABX94281 DNA DGENE  
TI New metal ion affinity peptide useful, when fused to a fusion partner polypeptide, for **protein purification** methods and to study protein-protein interactions and nucleic acid-protein interactions

IN Tchaga G S; Jokhadze G G  
PA (TCHA-I) TCHAGA G S.  
(JOKH-I) JOKHADZE G G.  
PI US 2002164718 A1 20021107 23p  
AI US 2001-858332 20010515  
PRAI US 1998-101867P 19980925  
US 1999-404017 19990923  
DT Patent  
LA English  
OS 2003-361747 [34]  
CR P-PSDB: ABU08448  
DESC DNA variant sequence encoding affinity purification peptide #3.  
AN ABX94281 DNA DGENE  
AB The present invention relates to metal ion affinity peptides, fusion proteins containing metal ion affinity peptides, and polynucleotide sequences encoding the fusion proteins. The presence of a metal ion

affinity peptide in a fusion protein allows purification of the fusion protein on a metal chelating resin. The method involves contacting a sample comprising a fusion protein with a **metal ion chelate resin** comprising a first metal ion, preferably a **hard metal ion** such as Fe<sup>3+</sup>, Ca<sup>2+</sup> or Al<sup>3+</sup> and eluting any resultant bound fusion protein from the resin. The resin comprises an immobilised Co<sup>2+</sup> ion. The method further comprises contacting the sample with a second immobilised metal ion affinity resin comprising a second immobilised metal ion and eluting any resultant bound fusion protein from the first and second resins. The second metal ion is an **intermediate metal ion** such as Cu<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup> or Co<sup>2+</sup>. The metal ion affinity peptide-tagged recombinant proteins are useful for the study of protein-protein interactions and nucleic acid molecule-protein interactions, using solid phase immobilised metal ion affinity chromatography (IMAC). They are also useful in high throughput systems which find use in massive parallel gene expression experiments, e.g. to determine the effect of an agent on synthesis of a protein or set of proteins, to analyse developmental stage-specific, or tissue-specific synthesis of a protein and to analyse the phosphorylation state of a protein. These methods find use in applications to characterise a protein of unknown identity or function, and in enzymatic reactions. ABX94279-ABX94283 represent DNA variants that encode affinity purification peptides.

L4 ANSWER 23 OF 29 DGENE COPYRIGHT 2005 The Thomson Corp on STN  
 AN ABX94280 DNA DGENE  
 TI New metal ion affinity peptide useful, when fused to a fusion partner polypeptide, for **protein purification** methods and to study protein-protein interactions and nucleic acid-protein interactions

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IN Tchaga G S; Jokhadze G G  
 PA (TCHA-I) TCHAGA G S.  
 (JOKH-I) JOKHADZE G G.  
 PI US 2002164718 A1 20021107 23p  
 AI US 2001-858332 20010515  
 PRAI US 1998-101867P 19980925  
 US 1999-404017 19990923  
 DT Patent  
 LA English  
 OS 2003-361747 [34]  
 CR P-PSDB: ABU08447  
 DESC DNA variant sequence encoding affinity purification peptide #2.  
 AN ABX94280 DNA DGENE  
 AB The present invention relates to metal ion affinity peptides, fusion proteins containing metal ion affinity peptides, and polynucleotide sequences encoding the fusion proteins. The presence of a metal ion affinity peptide in a fusion protein allows purification of the fusion protein on a metal chelating resin. The method involves contacting a sample comprising a fusion protein with a **metal ion chelate resin** comprising a first metal ion, preferably a **hard metal ion** such as Fe<sup>3+</sup>, Ca<sup>2+</sup> or Al<sup>3+</sup> and eluting any resultant bound fusion protein from the resin. The resin comprises an immobilised Co<sup>2+</sup> ion. The method further comprises contacting the sample with a second immobilised metal ion affinity resin comprising a second immobilised metal ion and eluting any resultant bound fusion protein from the first and second resins. The second metal ion is an **intermediate metal ion** such as Cu<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup> or Co<sup>2+</sup>. The metal ion affinity peptide-tagged recombinant proteins are useful for the study of protein-protein interactions and nucleic acid molecule-protein interactions, using solid phase immobilised metal ion affinity chromatography (IMAC). They are also useful in high throughput systems which find use in massive parallel gene expression experiments, e.g. to determine the effect of an agent on synthesis of a protein or set of proteins, to analyse developmental stage-specific, or



tissue-specific synthesis of a protein and to analyse the phosphorylation state of a protein. These methods find use in applications to characterise a protein of unknown identity or function, and in enzymatic reactions. ABX94279-ABX94283 represent DNA variants that encode affinity purification peptides.

L4 ANSWER 24 OF 29 DGENE COPYRIGHT 2005 The Thomson Corp on STN  
AN ABX94279 DNA DGENE  
TI New metal ion affinity peptide useful, when fused to a fusion partner polypeptide, for **protein purification** methods and to study protein-protein interactions and nucleic acid-protein interactions

IN Tchaga G S; Jokhadze G G

PA (TCHA-I) TCHAGA G S.

(JOKH-I) JOKHADZE G G.

PI US 2002164718 A1 20021107

23p

AI US 2001-858332 20010515

PRAI US 1998-101867P 19980925

US 1999-404017 19990923

DT Patent

LA English

OS 2003-361747 [34]

CR P-PSDB: ABU08446

DESC DNA variant sequence encoding affinity purification peptide #1.

AN ABX94279 DNA DGENE

AB The present invention relates to metal ion affinity peptides, fusion proteins containing metal ion affinity peptides, and polynucleotide sequences encoding the fusion proteins. The presence of a metal ion affinity peptide in a fusion protein allows purification of the fusion protein on a metal chelating resin. The method involves contacting a sample comprising a fusion protein with a **metal ion chelate resin** comprising a first metal ion, preferably a **hard metal ion** such as Fe<sup>3+</sup>, Ca<sup>2+</sup> or Al<sup>3+</sup> and eluting any resultant bound fusion protein from the resin. The resin comprises an immobilised Co<sup>2+</sup> ion. The method further comprises contacting the sample with a second immobilised metal ion affinity resin comprising a second immobilised metal ion and eluting any resultant bound fusion protein from the first and second resins. The second metal ion is an **intermediate metal ion** such as Cu<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup> or Co<sup>2+</sup>. The metal ion affinity peptide-tagged recombinant proteins are useful for the study of protein-protein interactions and nucleic acid molecule-protein interactions, using solid phase immobilised metal ion affinity chromatography (IMAC). They are also useful in high throughput systems which find use in massive parallel gene expression experiments, e.g. to determine the effect of an agent on synthesis of a protein or set of proteins, to analyse developmental stage-specific, or tissue-specific synthesis of a protein and to analyse the phosphorylation state of a protein. These methods find use in applications to characterise a protein of unknown identity or function, and in enzymatic reactions. ABX94279-ABX94283 represent DNA variants that encode affinity purification peptides.

L4 ANSWER 25 OF 29 DGENE COPYRIGHT 2005 The Thomson Corp on STN  
AN ABX94278 DNA DGENE  
TI New metal ion affinity peptide useful, when fused to a fusion partner polypeptide, for **protein purification** methods and to study protein-protein interactions and nucleic acid-protein interactions

IN Tchaga G S; Jokhadze G G

PA (TCHA-I) TCHAGA G S.

(JOKH-I) JOKHADZE G G.

PI US 2002164718 A1 20021107

23p

AI US 2001-858332 20010515

PRAI US 1998-101867P 19980925

US 1999-404017 19990923

DT Patent  
LA English  
OS 2003-361747 [34]  
CR P-PSDB: ABU08445  
DESC Vector pHAT-EK containing cDNA encoding recombinant enterokinase (EK).  
AN ABX94278 DNA DGENE  
AB The present invention relates to metal ion affinity peptides, fusion proteins containing metal ion affinity peptides, and polynucleotide sequences encoding the fusion proteins. The presence of a metal ion affinity peptide in a fusion protein allows purification of the fusion protein on a metal chelating resin. The method involves contacting a sample comprising a fusion protein with a **metal ion chelate resin** comprising a first metal ion, preferably a **hard metal ion** such as Fe<sup>3+</sup>, Ca<sup>2+</sup> or Al<sup>3+</sup> and eluting any resultant bound fusion protein from the resin. The resin comprises an immobilised Co<sup>2+</sup> ion. The method further comprises contacting the sample with a second immobilised metal ion affinity resin comprising a second immobilised metal ion and eluting any resultant bound fusion protein from the first and second resins. The second metal ion is an **intermediate metal ion** such as Cu<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup> or Co<sup>2+</sup>. The metal ion affinity peptide-tagged recombinant proteins are useful for the study of protein-protein interactions and nucleic acid molecule-protein interactions, using solid phase immobilised metal ion affinity chromatography (IMAC). They are also useful in high throughput systems which find use in massive parallel gene expression experiments, e.g. to determine the effect of an agent on synthesis of a protein or set of proteins, to analyse developmental stage-specific, or tissue-specific synthesis of a protein and to analyse the phosphorylation state of a protein. These methods find use in applications to characterise a protein of unknown identity or function, and in enzymatic reactions. The present sequence represents vector pHAT-EK.

L4 ANSWER 26 OF 29 IFIPAT COPYRIGHT 2005 IFI on STN

AN 10673176 IFIPAT;IFIUDB;IFICDB

TI METHODS AND COMPOSITIONS FOR PROTEIN PURIFICATION

INF Jokhadze; George G., Mountain View, CA, US

Tchaga; Grigoriy S., Newark, CA, US

IN Jokhadze George G; Tchaga Grigoriy S

PAF Unassigned

PA Unassigned Or Assigned To Individual (68000)

AG BOZICEVIC, FIELD & FRANCIS (BD BIOSCIENCES), 200 MIDDLEFIELD ROAD, SUITE 200, MENLO PARK, CA, 94025, US

PI US 2004180415 A1 20040916

AI US 2004-762588 20040121

RLI US 2001-858332 20010515 CONTINUATION-IN-PART PENDING

PRAI US 2003-441804P 20030121 (Provisional)

FI US 2004180415 20040916

DT Utility; Patent Application - First Publication

FS CHEMICAL

APPLICATION

PARN This application is a continuation-in-part of U.S. patent application Ser. No. 09/858,332, filed May 15, 2001, which application is incorporated herein by reference in its entirety. This application also claims the benefit of U.S. Provisional Patent Application No. 60/441,804, filed Jan. 21, 2003; which application is incorporated herein by reference in its entirety.

CLMN 17

GI 2 Figure(s).

FIG. 1 depicts an exemplary **protein purification** scheme.

FIG. 2 depicts gel electrophoresis analysis of various fractions from the purification scheme described in Example 1 and shown in FIG. 1.

OF 29 IFIPAT COPYRIGHT 2005 IFI on STN

AB The present invention provides methods of purifying proteins that include a metal ion affinity peptide. The methods generally involve contacting a fusion protein that includes a metal ion affinity peptide with at least two different metal ion chelating resins. In certain representative embodiments, the methods include contacting a fusion protein with a first **metal ion chelate resin** having a first immobilized metal ion; eluting any bound protein from the first **metal ion chelate resin**, to produce an eluate; contacting the eluate with a second **metal ion chelate resin** having a second immobilized metal ion; and eluting any bound protein from the second **metal ion chelate resin**. Also provided are kits for use in practicing the subject methods. The subject methods find use in a variety of **protein purification** applications.

CLMN 17 2 Figure(s).

FIG. 1 depicts an exemplary **protein purification** scheme.

FIG. 2 depicts gel electrophoresis analysis of various fractions from the purification scheme described in Example 1 and shown in FIG. 1.

L4 ANSWER 27 OF 29 USPATFULL on STN

AN 2004:233344 USPATFULL

TI Methods and compositions for **protein purification**

IN Tchaga, Grigoriy S., Newark, CA, UNITED STATES

Jokhadze, George G., Mountain View, CA, UNITED STATES

PI US 2004180415 A1 20040916

AI US 2004-762588 A1 20040121 (10)

RLI Continuation-in-part of Ser. No. US 2001-858332, filed on 15 May 2001, PENDING

PRAI US 2003-441804P 20030121 (60)

DT Utility

FS APPLICATION

LREP BOZICEVIC, FIELD & FRANCIS (BD BIOSCIENCES), 200 MIDDLEFIELD ROAD, SUITE 200, MENLO PARK, CA, 94025

CLMN Number of Claims: 17

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 1687

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides methods of purifying proteins that include a metal ion affinity peptide. The methods generally involve contacting a fusion protein that includes a metal ion affinity peptide with at least two different metal ion chelating resins. In certain representative embodiments, the methods include contacting a fusion protein with a first **metal ion chelate resin** having a first immobilized metal ion; eluting any bound protein from the first **metal ion chelate resin**, to produce an eluate; contacting the eluate with a second **metal ion chelate resin** having a second immobilized metal ion; and eluting any bound protein from the second **metal ion chelate resin**. Also provided are kits for use in practicing the subject methods. The subject methods find use in a variety of **protein purification** applications.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 28 OF 29 USPATFULL on STN

AN 2002:294675 USPATFULL

TI Metal ion affinity tags and methods for using the same

IN Tchaga, Grigoriy S., Newark, CA, UNITED STATES

Jokhadze, George G., Mountain View, CA, UNITED STATES

PI US 2002164718 A1 20021107

AI US 2001-858332 A1 20010515 (9)

RLI Continuation-in-part of Ser. No. US 1999-404017, filed on 23 Sep 1999,  
ABANDONED  
PRAI US 1998-101867P 19980925 (60)  
DT Utility  
FS APPLICATION  
LREP BOZICEVIC, FIELD & FRANCIS LLP, 200 MIDDLEFIELD RD, SUITE 200, MENLO  
PARK, CA, 94025  
CLMN Number of Claims: 28  
ECL Exemplary Claim: 1  
DRWN 8 Drawing Page(s)  
LN.CNT 1484

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides metal ion affinity peptides, fusion  
proteins comprising metal ion affinity peptides, and polynucleotides  
encoding the fusion proteins. The invention further provides recombinant  
vectors comprising subject polynucleotides, and host cells comprising  
the recombinant vectors. The invention further provides methods and kits  
for purifying a fusion protein comprising a metal ion affinity peptide.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 29 OF 29 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN  
AN 2003-361747 [34] WPIDS  
CR 2004-675606 [66]  
DNC C2003-095381  
TI New metal ion affinity peptide useful, when fused to a fusion partner  
polypeptide, for **protein purification** methods and to  
study protein-protein interactions and nucleic acid-protein interactions.

DC B04 D16

IN JOKHADZE, G G; TCHAGA, G S

~~PA (JOKH-I) JOKHADZE G G; (TCHA-I) TCHAGA G S~~

CYC 1

PI US 2002164718 A1 20021107 (200334)\* 23

ADT US 2002164718 A1 Provisional US 1998-101867P 19980925, CIP of US  
1999-404017 19990923, US 2001-858332 20010515

PRAI US 1998-101867P 19980925; US 1999-404017 19990923;  
US 2001-858332 20010515

AN 2003-361747 [34] WPIDS

CR 2004-675606 [66]

AB US2002164718 A UPAB: 20041015

NOVELTY - A metal ion affinity peptide (I), is new.

DETAILED DESCRIPTION - A new metal ion affinity peptide (I) consists  
of formula 1,2

(His-X1-X2)n1-(His-X3-X4-X5)n2-(His-X6)n3 (1)

X1 and X2 = independently an amino acid with an aliphatic or an amide  
side chain;

X1 and X2 = independently an amino acid with an aliphatic or an amide  
side chain;

X3, X4 and X5 = independently an amino acid with a basic side chain  
(except His) or an acidic side chain;

X6 = an amino acid with an aliphatic or an amide side chain;

n1 and n2 = independently 1 - 3; and

n3 = 1 - 5;

(His-Asn)n (2)

n = 3 - 10

(His-X1-X2)n (3)

X1 and X2 = an amino acid having an acidic side chain; and

n = 3 - 10.

INDEPENDENT CLAIMS are also included for the following:

(1) a fusion protein comprising a polypeptide fused at its amino- or  
carboxy-terminus to (I);

(2) an isolated polynucleotide (II) comprising a nucleotide sequence  
encoding (I);

(3) a recombinant vector (III) comprising (II);

- (4) a recombinant host cell comprising (III); and
- (5) a kit for purifying a protein, comprising (III) and a metal ion affinity resin.

USE - The metal ion affinity peptide in a fusion protein allows purification of the fusion protein on a metal chelating resin. The method involves contacting a sample comprising a fusion protein with a **metal ion chelate resin** comprising a first metal ion, preferably a **hard metal ion** such as  $\text{Fe}^{3+}$ ,  $\text{Ca}^{2+}$  and  $\text{Al}^{3+}$  and eluting any resultant bound fusion protein from the resin. The resin comprises an immobilized  $\text{Co}^{2+}$  ion. The method further comprises contacting the sample with a second immobilized metal ion affinity resin comprising a second immobilized metal ion and eluting any resultant bound fusion protein from the first and second resins. The second metal ion is an **intermediate metal ion** such as  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Co}^{2+}$  (claimed). The metal ion affinity peptide-tagged recombinant proteins are useful for the study of protein-protein interactions and nucleic acid molecule-protein interactions, using solid phase immobilized metal ion affinity chromatography (IMAC). They are also useful in high throughput systems which find use in massive parallel gene expression experiments, e.g. to determine the effect of an agent on synthesis of a protein or set of proteins, to analyze developmental stage-specific, or tissue-specific synthesis of a protein and to analyze the phosphorylation state of a protein. These methods find use in applications to characterize a protein of unknown identity or function, and in enzymatic reactions.

Dwg.0/6